Original Article

Triple Negative Breast Cancer at the Jos University Teaching Hospital

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Abstract

Background: Breast cancer remains the leading cause of cancer morbidity and mortality in women worldwide. The treatment of breast cancer has drifted from radical mastectomy, to conservative treatment owing to the presence of micro metastasis at even earlier stages of the disease. These novel medical treatments are hinged on characterizing breast cancer at the molecular level. Estrogen receptor (ER), Progesterone receptor (PR), and Human epidermal growth factor 2 receptor (HER2) are the commonest molecular-markers used. Failure to stain for these three receptors, earns a breast cancer the triple negative designation, an aggressive type of the disease with the worst prognosis. **Materials and Methods:** All histologically confirmed cases of breast cancer at Jos University Teaching Hospital, between 2010 and 2012, were retrieved, and their histological type and grade documented. Using the Avidin-biotin method, the status of each case was established for ER, PR, and HER2. Cases with negative staining for these three antibodies were further stained with CK5/6, a basal biomarker for Triple Negative Breast Cancer (TNBC). Statistical softwares were used to analyse the data and present in tables. **Results:** Sixty-three cases of female breast cancers met the inclusion criteria. The age range was 28-74 years with mean age of 46.7+11.1 years. Invasive carcinoma (no special type) was the majority of histological type of breast cancer, accounting for 54(85.7%) cases. Histological grades 1, 2 and 3, recorded 18(28.6%), 29(46.0%), and 16(25.4%), cases respectively. TNBC accounted for 26 (41.3%) cases. Fifteen (57.7%) cases of TNBC stained positively for CK 5/6. **Conclusion:** Indicators of worse prognosis which includes lower hormone receptor status and high percentage of TNBC, as well as greater number of basal subtype of TNBC was exhibited by the study population.

Keywords: Breast, cancer, Jos, negative, triple

Introduction

Breast cancer remains the leading cause of cancer morbidity and mortality in women worldwide.[1-6]

The burden of breast cancer is increasing in developing countries. By 2020, it is estimated that 70% of new cases of cancer would occur in the developing countries, and majority of these cases would be cancer of the breast.[7,8] Although much research work has been undertaken, it is still mysterious why "a neoplasm arising in an organ readily accessible to self-examination and clinical diagnosis, continues to exert such a heavy toll."[1]

In both Africans and African American women, late presentation has hindered the management of breast cancer, with up to 90% of cases diagnosed at stage III-IV of the disease in Africa.[7,9-12] This has been attributed to ignorance of the nature of disease, economic, sociocultural factors, lack of screening programs for breast cancer, among many other limiting factors.[7,9-12]

The approach toward clinical assessment and management of breast cancer has substantially changed over the past few decades. Radical mastectomy is no longer fashionable as breast cancer is now considered a systematic disease from the outset, seeing that most patients with early breast cancer present with metastasis.[13] Furthermore, the aggressiveness of local treatment does not reflect in patient's survival or risk of metastasis.[14] This could be explained by blood-borne micrometastasis being already present at the time of initial

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Access this article online

Quick Response Code:

Website: www.atpjournal.org

DOI: 10.4103/atp.atp_14_17

How to cite this article: Mandong BM, Emmanuel I, Vandi KB, Yakubu D. Triple negative breast cancer at the Jos University Teaching Hospital. Ann Trop Pathol 2017;8:20-4.

diagnosis. Therefore, the trend is toward a more conservative approach to breast cancer surgery and use of medical therapy. Medication used as adjuvant therapy has clearly proven beneficial presumably by eradicating metastatic deposits.[14]

Researchers have established the clinical heterogeneity of breast cancer, with each subtype responding differently to treatment.[15,16] It is, therefore, worthwhile to isolate, assess, and manage each patient with the disease as an entity.[17] Immunohistochemistry as an adjunct in assessing molecular pattern of breast cancer, has contributed to prognostication of the disease. Currently, three biomarkers are commonly used to classify breast cancer. These are the estrogen Receptor (ER), the progesterone receptor (PR), and the human epidermal growth factor receptor 2 (HER-2). Assessing these markers help to select patients appropriately for treatment targeted on these major molecular pathways of the disease.[18,19]

A breast cancer that fails to express ER and PR, and has absence of HER-2 overexpression is designated "Triple Negative."[20] Triple negative breast cancer (TNBC) compared with other groups of breast cancer describes a subtype of this malignant disease with worse prognosis and dismal survival outcomes.[21]

TNBC constitutes one of the most challenging groups of breast cancers.[20] It is associated with unfavorable and aggressive clinicopathological features including onset at a younger age, high grade, pushing borders, poorer Nottingham prognostic index, much frequent node positivity, higher incidence of recurrence, distant metastasis, and poorer survival.[17,22]

The complexity of breast cancer deepens further as TNBC is described also as a heterogeneous disease.[23] Molecular panels such as basal keratin and other markers (Ck5/6, Ck14, Ck17, 34BE112, CD117, EGFR, P53, and SMA) have been used in subtyping TNBCs into two groups.[24] Positivity for these stains describes the basal, to differentiate it from the nonbasal subtype. The former constitute the majority of TNBCs and has a worse prognosis than the latter.[25]

The purpose of this study is to determine the proportion of TNBCs in 63 patients who met the criteria for immunohistochemical studies and to further subclassify the triple negative tumors into basal and nonbasal types based on staining for CK 5/6, as well as their relationship to age, histological type, and histological grade.

Materials and Methods

Sixty-three cases of histologically confirmed breast cancers with complete biodata were retrieved from the records (2010–2012) of the cancer registry of the Department of Histopathology, Jos University Teaching Hospital.

All cases with sufficient biodata were included, while those with unsuitable tissue blocks were excluded from the study.

These cases were histologically subtyped and graded using the modified Bloom-Richardson system.[1,26]

These cases were subjected to immunohistochemistry with immunoperoxidase (Avidin-Biotin complex technique) staining for estrogen, progesterone, and HER-2 receptors.[27,28] The antibody clone for ER, PR, and HER-2 used were (dilution 1:100): NCL-L-ER; L-PGR; and NCL-L-CB11, respectively, all from Leica.

Standardized human breast tissues (tissues with known positivity, for ER, PR, HER, and CK 5/6) was used as a control.

The immunohistochemistry procedure used is further described. Paraffin-embedded tissue was cut at 3 microns thick and allowed to cool on a hot plate for an hour. Sections were taken into various stages of processing which included: water, xylene, alcohol, and finally water. Antigen retrieval was performed using citric acid solution (PH 6.0) in a microwave at power 100 for 15 min.

Sections were equilibrated by gently displacing the hot citric acid with running tap water for 3 min. Peroxidase was blocked in tissue using peroxidase block for 15 min. Sections were washed for 2 min with phosphate buffered saline (PBS) mixed with Tween 20. Sections were then blocked with Novocastra protein block for 15 min. Sections were washed for 2 min with PBS. Sections were incubated with primary antibody for 45 min. Sections were washed for 5 min with PBS. The secondary antibody was added for 15 min. Sections were washed twice with PBS. The polymer was added for 15 min. Sections were washed twice with PBS and diaminobenzidine (DAB) (diluted 1/100 with DAB substrate) for 5 min.

Sections were washed with water and counterstained for 2 min with hematoxylin. Sections were washed, dehydrated, cleared, and mounted.

Fluorescent *in situ* hybridisation, was not be done on the 2 cases of breast cancer that were + 2 for HER-2. These cases, however, were not triple negative as they were positive for hormone receptor (ER and PR).

The scoring system for ER and PR status was based on J score which score the percentage of tumor cells with nuclear staining: Score 0, no stained cells; Score 1+, stained cells ≤1%; Score 2+, 1% <stained cells <10%; and Score 3+, stained cells ≥10%. Scores 0 was classified as negative; Scores 1 and 2 as intermediate; and Score 3 as positive.[27,28]

HER-2 staining was scored based on membrane staining pattern (intensity and completeness)-Hercept test: score 0, when no staining is observed, or is seen in <10% of tumor cells; Score 1+, when staining is faint/barely seen in >10% of tumor cells; Score 2+, when a weak/moderate complete staining is observed in >10% of tumor cell; and Score 3+, when a strong complete staining is detected in >10% of tumor cells.[28] Score 0 and 1+ were classified as negative; Score 2+ as inconclusive (weakly positive); and Score 3+ as positive.[28,29]

Specimens that fail to be positive for ER, PR, and HER-2 earned the triple negative designation.

They were further stained using antibodies for CK5/6. A 10% complete staining for the antibody was considered positive (CK5/6 is frequently used to define basal subtype of TNBC, and it is also the most available marker in our locality).[20,29]

The LEICA DM 500 (LEICA ICC 50 HD) microscope was used to review the histological slides and acquire photomicrographs [Figure 1].

Results

Within the period of the study, a total of 96 cases of breast cancers were diagnosed histologically at the Jos University Teaching Hospital. All cases were female breast cancers. A total of 63 (65.63%) cases of these were included in the study, as they had adequate records and were sufficient for staining with the four immunomarkers.

The age distribution is presented in Table 1. The age range was 28–74 years. The mean age at diagnosis was 46.7 + 11.1 years. The mean age for TNBC positive and CK 5/6 positive cases was 45.2 + 9.2 and 48.3 + 10.4, respectively. The median age was 46 years.

There were 26 (41.3%) cases of TNBCs. Cytokeratin 5/6 was used to subtype the 26 cases of TNBC into basal subtype which stains positively, and nonbasal subtype which do not stain. Up to 15 (57.7%) TNBC cases stained positively for CK 5/6.

Invasive ductal carcinoma (NST) constituted the vast majority of histological types of breast cancer, accounting for 54 (85.7%) cases [Table 2]. The TNBC was predominantly invasive ductal carcinomas, 24 (92.3%) cases. The remaining 2 (7.7%) cases of TNBC were mucinous carcinomas. Cytokeratin 5/6 positive TNBC was 93.3% (14 cases) invasive ductal carcinoma and 6.7% (1 case) mucinous carcinoma.

The histological Grades 1, 2, and 3 recorded 18 (28.6%), 29 (46.0%), and 16 (25.4%) cases, respectively [Table 3]. The association between histological grade and TNBC status for Grades 1, 2, and 3, were 8 (30.8%), 13 (50.0%), and 5 (19.2%) of cases, respectively. Cytokeratin 5/6 positive TNBC recorded 4 (26.7%), 8 (53.3%), and 3 (20.0%) cases for Grade 1, 2, and 3, respectively.

Table 1: Distribution of female breast cancer according to age

Age group	Frequency	Frequency of TNBC cases	Frequency of CK 5/6 positive cases
20-29	2	1	0
30-39	17	5	1
40-49	16	9	6
50-59	16	9	7
60-69	10	2	1
70-79	2	-	-
Total	63	26	15

TNBC: Triple negative breast cancer, CK: Cytokeratin

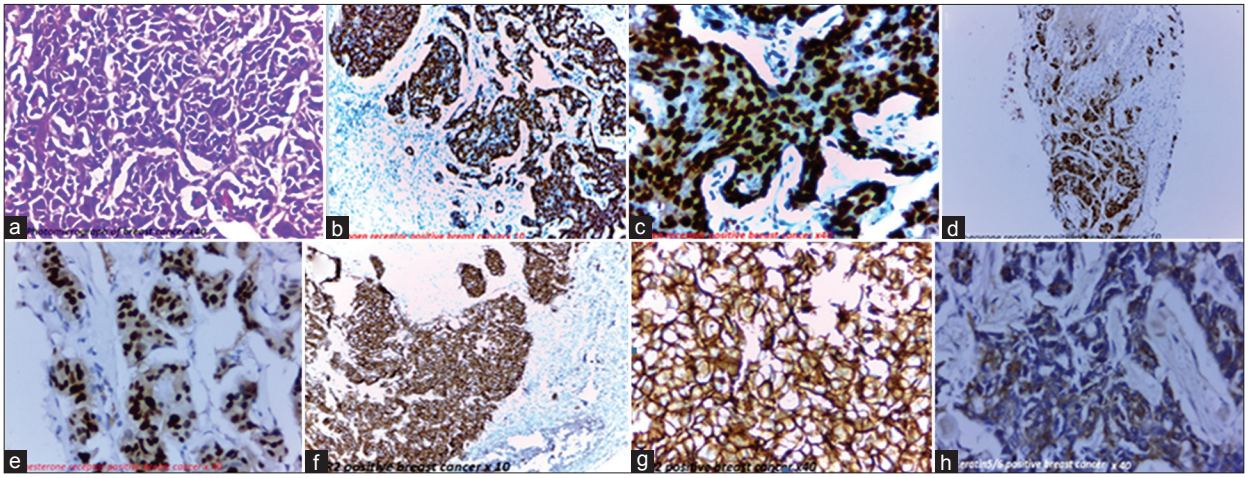

Figure 1: (a) Photomicrographs of Grade 2 breast cancer staining positively with H/E, estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2, and cytokeratin 5/6 stains (H and E). (b) Photomicrographs of Grade 2 breast cancer staining positively with H/E, estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2, and cytokeratin 5/6 stains. Strong nuclear staining for estrogen receptor. (c) Photomicrographs of Grade 2 breast cancer staining positively with H/E, estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2, and cytokeratin 5/6 stains. Strong nuclear staining for estrogen receptor. (d) Photomicrographs of Grade 2 breast cancer staining positively with H/E, estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2, and cytokeratin 5/6 stains. Strong nuclear staining for progesterone receptor. (e) Photomicrographs of Grade 2 breast cancer staining positively with H/E, estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2, and cytokeratin 5/6 stains. Strong nuclear staining for progesterone receptor. (f) Photomicrographs of Grade 2 breast cancer staining positively with H/E, estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2, and cytokeratin 5/6 stains. Strong nuclear staining for progesterone receptor. (g) Photomicrographs of Grade 2 breast cancer staining positively with H/E, estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2, and cytokeratin 5/6 stains. Intense and complete membrane staining for human epidermal growth factor receptor 2. (h) Photomicrographs of Grade 2 breast cancer staining positively with H/E, estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2, and cytokeratin 5/6 stains. Strong cytoplasmic staining for cytokeratin 5/6

Table 2: Distribution of female breast cancer according to histological type

Histological type	Frequency (%)	Frequency of TNBC cases (%)	Frequency of CK 5/6 positive cases (%)
Invasive ductal carcinoma (NOS)	54 (85.7)	24 (92.3)	14 (93.3)
Invasive lobular carcinoma	2 (3.2)	-	-
Ductal carcinoma *in situ*	1 (1.6)	-	-
Papillary carcinoma	1 (1.6)	-	-
Tubular carcinoma	1 (1.6)	-	-
Mucinous carcinoma	4 (6.3)	2 (7.7)	1 (6.7)
Total	63 (100)	26 (100)	15 (100)

NOS: Nonotherwise specified, TNBC: Triple negative breast cancer, CK: Cytokeratin

Table 3: Distribution of female breast cancer according to histological grade

Grade	Frequency (%)	Frequency of TNBC cases (%)	Frequency of CK 5/6 positive cases (%)
1	18 (28.6)	8 (30.8)	4 (26.7)
2	29 (46.0)	13 (50.0)	8 (53.3)
3	16 (25.4)	5 (19.2)	3 (20.0)
Total	63 (100.0)	26 (100.0)	15 (100.0)

TNBC: Triple negative breast cancer, CK: Cytokeratin

Discussion

We present 63 cases of histologically confirmed invasive breast cancer which made the requirements for immunohistochemistry for ER, PR, and HER-2.

The mean age of these patients of 46.7% is similar to previous studies in our center and other centers in Nigeria.[30-35] Breast cancer appears earlier in Africa and African Americans.[33,34] This finding of earlier occurrence of breast cancer in Africans than in Caucasians, has been attributed to a shorter life expectancy in the former than in the later.[36,37] As fewer Africans attain an elderly age, only cancers occurring at an earlier age are seen.

Triple Negative Breast Cancer in this study showed a lower mean age (45.2 + 9.2) than that of entire cases of breast cancers analyzed. This is consistent with reports by other researchers who found TNBC to be a malignancy with onset at a younger age.[17,22] However, a higher mean age than the general mean was seen for the CK 5/6 positive TNBC.

We found that invasive ductal carcinoma (nonotherwise specified) was the predominant histological type accounting for 85.7% of cases. This finding is corroborated by other researchers across the globe.[20,38-41]

Our findings with regard the relative frequency of TNBC to other molecular subtypes of breast cancer are similar to a previous study in our center and other African reports.[35,42-44] Studies have shown that Caucasian women have higher frequency of this cancer than their African counterpart.[17,22,41,45]

It is known that majority of TNBC arise in women with BRCA1 mutation.[22,46-48] However, our study did not access the status of BRCA1.

Majority of the TNBC were Grade 1 and 2 tumors, a finding that is not consistent with previous reports.[40,41] These earlier studies show the clustering of the majority of TNBCs in Grade 3, depicting the correlation between tumor aggressiveness and worse grade. The variance seen in this study may be as a result of a relatively low sample size.

Fifteen of the twenty-six cases of TNBC were positive for Cytokeratin 5/6 (CK5/6). This special subtype (basal subtype) is generally aggressive with a high grade.[15,20,24,29,49] Eleven of the fifteen cases were of Grade 2 and 3. These cancers have been demonstrated to be aggressive and metastasize to viscera and brain more frequently than other histological variants.[15,20,24,29,49]

Conclusion

Indicators of worse prognosis which includes lower hormone receptor status and high percentage of TNBC, as well as greater number of basal subtype of TNBC was exhibited by the study population. Further studies in this area are necessary in our environment, to assess the survival of women with TNBCs and it is basal and nonbasal subtype.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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